

# Current and Future Perspectives on ADAMTS13 and Thrombotic Thrombocytopenic Purpura

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## Abstract

Thrombotic thrombocytopenic purpura (TTP) is a rare, relapsing, and life-threatening disorder with an annual incidence of 10 cases per million people. TTP is a thrombotic microangiopathy characterized by severe thrombocytopenia, microangiopathic hemolytic anemia, and organ ischemia. The disease is caused by a severe deficiency of the enzyme ADAMTS13 (*a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13*), which can either be acquired, mainly by autoantibodies targeting ADAMTS13, or congenital due to mutations in the *ADAMTS13* gene. Thanks to the establishment of national registries worldwide, fundamental and translational research, major advances have been made on the diagnosis, treatment, and fundamental understanding of TTP, since the description of the first TTP case almost 100 years ago. The introduction of therapeutic plasma exchange in the 1970s has significantly improved patient survival, but novel diagnostic assays, targeted treatments (rituximab, caplacizumab, recombinant ADAMTS13), and the unraveling of both ADAMTS13 function and TTP pathophysiology should help to further improve the patients' quality of life. However, differential diagnosis of TTP remains challenging and still a lot of questions remain unanswered to completely understand this rare and devastating disease.

## Keywords

- ▶ ADAMTS13
- ▶ thrombotic thrombocytopenic purpura
- ▶ treatment
- ▶ diagnosis

## Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening thrombotic microangiopathy (TMA) with a high relapse rate (20–50%) and is characterized by a severe thrombocytopenia (generally  $<30 \times 10^9/L$ ), a microangiopathic anemia (schistocytes on the blood smear), and organ ischemia.<sup>1–9</sup> The annual prevalence of TTP is approximately 10 cases per million people and its annual incidence is approximately one new case per million people.<sup>7,10</sup> Although the first case of TTP was already described in 1924 by Moschcowitz in a 16-year-old girl,<sup>11</sup> it took until 1997 to link TTP with a severe deficiency of the von Willebrand factor cleaving-protease (VWF-CP; activity  $<10\%$  or 10 UI/dL),<sup>12–14</sup> which was later characterized as the 13th member of the ADAMTS protein family (*a dis-*

*integrin and metalloprotease with thrombospondin type 1 repeats, member 13*) in 2001.<sup>15–19</sup> The interest of clinicians and scientists to perform fundamental and translation research on ADAMTS13 and TTP and the establishment of national registries worldwide have significantly improved the fundamental understanding of ADAMTS13 and the diagnosis and therapeutic management of TTP patients (▶ Fig. 1).<sup>1–5,7–9,20–23</sup> However, still a lot of questions remain unanswered. In this review the current understanding of ADAMTS13 and TTP is discussed.

## ADAMTS13

ADAMTS13 is an important enzyme in the blood, responsible to regulate the size of the VWF multimers. The gene of

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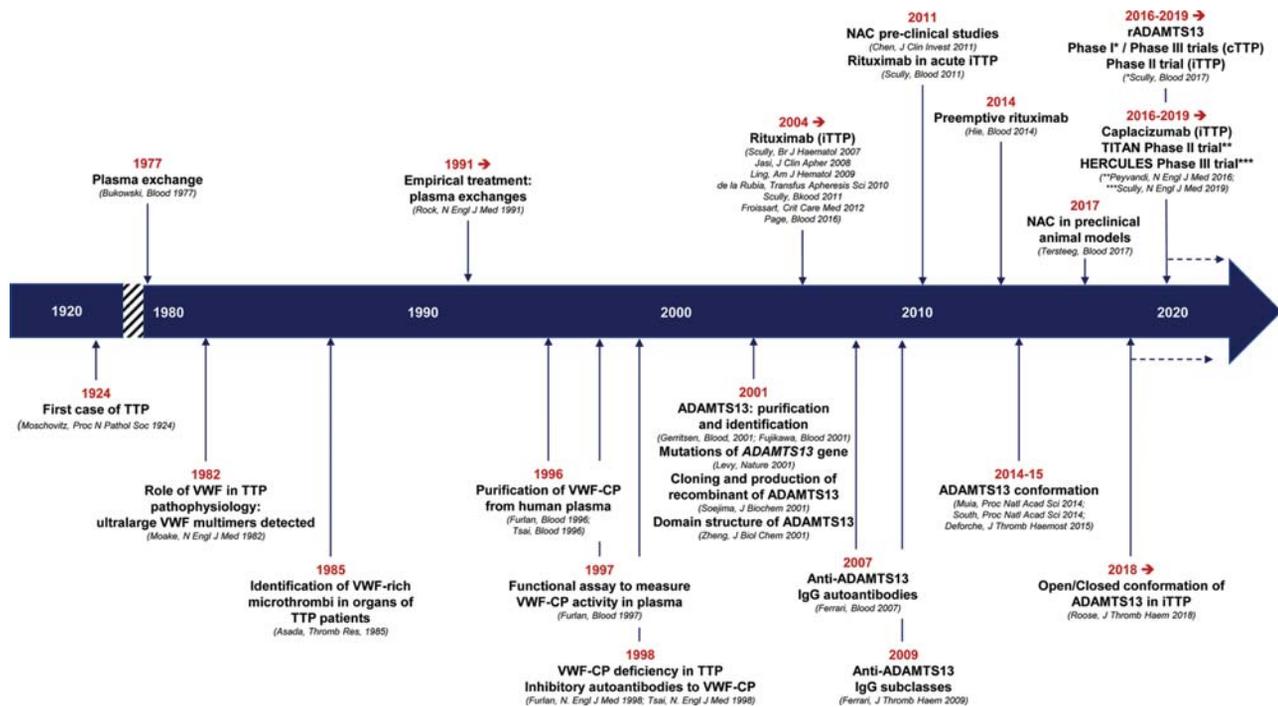
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**Fig. 1** Timeline of ADAMTS13 discovery and thrombotic thrombocytopenic purpura treatment. cTTP, congenital TTP; IgG, immunoglobulin G; iTTP, immune-mediated TTP; NAC, N-acetylcysteine; rADAMTS13, recombinant ADAMTS13; TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor; VWF-CP, VWF-cleaving protease.

ADAMTS13 is encoded by 29 exons located on chromosome 9q34.<sup>15,17</sup> This enzyme, consisting of 1,427 amino acids, is mainly synthesized in the hepatic stellate cells of the liver and results in a circulating plasma concentration of approximately 1 µg/mL with a half-life of 2 to 4 days.<sup>16,24–26</sup>

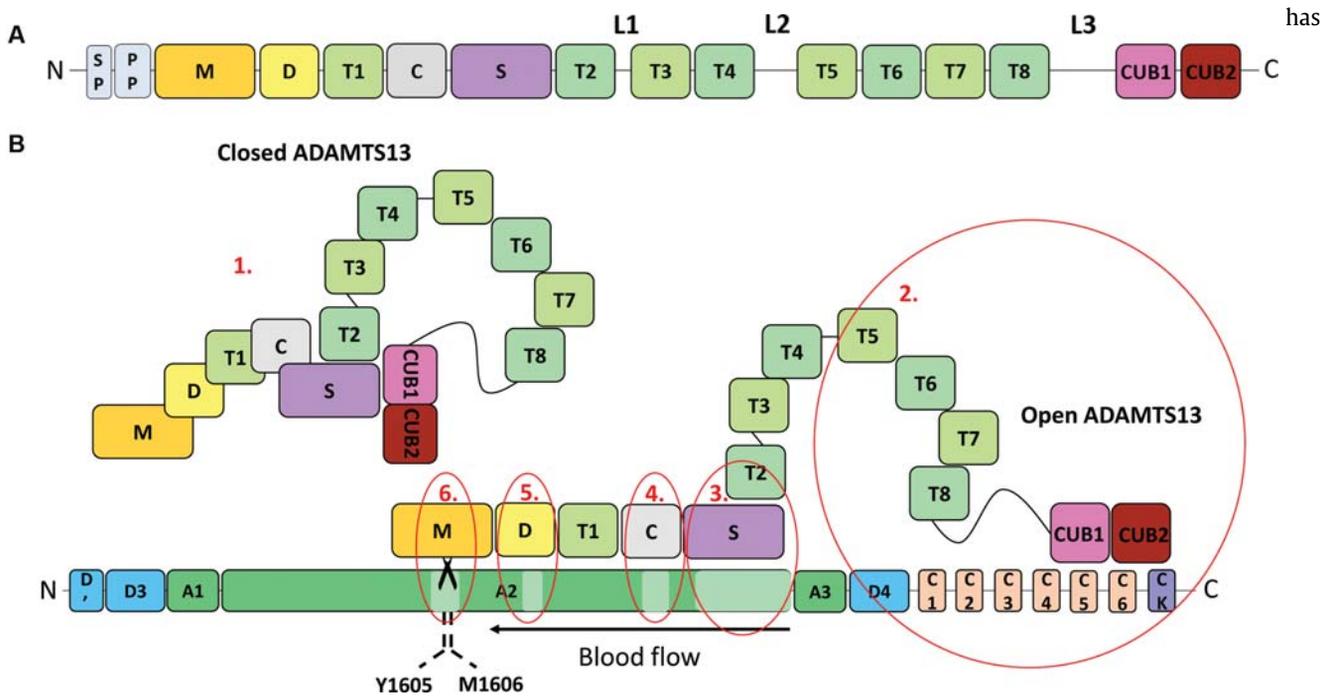
ADAMTS13 consists of several domains: a signal peptide (SP), a propeptide (PP), a metalloprotease domain (M), a disintegrin-like domain (D), a first thrombospondin type-1 repeat (T1), a cysteine-rich domain (C), a spacer domain (S), seven additional thrombospondin type-1 repeats (T2–T8), and two CUB domains (–Fig. 2A).<sup>18</sup> The C-terminal part of ADAMTS13 contains three linker (L) regions located between T2 and T3 (L1), T4 and T5 (L2), and T8 and CUB1 (L3; –Fig. 2A), which introduce a lot of flexibility in the molecule,<sup>27</sup> making it difficult to resolve the crystal structure of full-length ADAMTS13. Each domain of ADAMTS13 has a role to regulate the multimeric size of the VWF multimers through a molecular zipper mechanism (–Fig. 2B) to avoid spontaneous platelet aggregation.<sup>28</sup> The proteolysis of VWF by ADAMTS13 is dependent on conformational changes of both proteins.<sup>27,29–33</sup> VWF, a multimeric glycoprotein stored in the Weibel–Palade bodies of endothelial cells or α-granules of platelets, is secreted in a globular form.<sup>34,35</sup> Upon shear stress, VWF multimers unravel thereby exposing their ADAMTS13 binding and cleavage site Tyr1605–Met1606 (located in the A2 domain of VWF).<sup>29,31</sup> Under normal physiological conditions, ADAMTS13 circulates in a closed conformation where spacer and T8–CUB2 domains interact.<sup>27,32,33</sup> An initial interaction between VWF and the T5–CUB2 domains allosterically activates ADAMTS13, inducing an open ADAMTS13 conformation in which the interaction

between spacer and CUB domains is abrogated.<sup>27,30</sup> VWF substrate recognition by ADAMTS13 is regulated through exosites present in the spacer domain, cysteine-rich domain, and disintegrin-like domain. Recently, it has been shown by the group of Prof. Crawley that the exosites in the disintegrin-like, cysteine-rich, and spacer domains primarily contribute to the substrate recognition (Km). However, the exosite located in the disintegrin-like domain also has a major influence on the catalytic efficiency (kcat) of VWF proteolysis.<sup>36</sup> Indeed, binding of the disintegrin-like domain to VWF allosterically activates the otherwise latent metalloprotease domain, giving VWF access to the active-site cleft, resulting in the proteolysis of VWF.<sup>36</sup>

When the ADAMTS13 activity is severely impaired, the VWF multimers will start to accumulate to which platelets can bind and form VWF/platelet-rich thrombi that cause microvascular occlusion, resulting in the rare and life-threatening disease TTP.<sup>6</sup>

### Thrombotic Thrombocytopenic Purpura

The severe deficiency of ADAMTS13 is pathognomonic of TTP and mostly occurs in adults (90% of all cases).<sup>7,21,37</sup> The mechanism of severe ADAMTS13 deficiency can either be acquired (95% of all TTP cases) and mainly immune-mediated (iTTP) due to the presence of anti-ADAMTS13 autoantibodies<sup>14,38–40</sup> or congenital (cTTP, Upshaw–Schulman syndrome, OMIM 274.150; 5% of all TTP cases) and linked to biallelic mutations of the *ADAMTS13* gene.<sup>15,22,41–45</sup> At presentation, TTP may either be idiopathic (50% of TTP patients) or associated to a pre-existing condition (50% of TTP patients; infections, autoimmune diseases, human



**Fig. 2** ADAMTS13 domain organization and molecular zipper mechanism. (A) ADAMTS13 domain organization. ADAMTS13 consists of several domains: a signal peptide (SP), a propeptide (PP), a metalloprotease domain (M), a disintegrin-like domain (D), a first thrombospondin type-1 repeat (T1), a cysteine-rich domain (C), a spacer domain (S), seven additional thrombospondin type-1 repeats (T2–T8), and 2 CUB domains. The C-terminal part of ADAMTS13 also contains three linker (L) regions located between: T2 and T3 (L1); T4 and T5 (L2); T8 and CUB1 (L3). (B) Molecular zipper mechanism of the proteolysis of von Willebrand factor (VWF) by ADAMTS13. ADAMTS13 normally circulates in a closed conformation where spacer and T8-CUB2 domains interact (1.). ADAMTS13 will become allosterically activated due to an initial interaction between D4-CK domains of VWF and its T5-CUB2 domains, thereby inducing an open ADAMTS13 conformation in which the interaction between spacer and CUB domains is abrogated (2.). Due to shear stress of the blood, VWF multimers unravel thereby exposing their ADAMTS13 binding and cleavage sites Tyr1605-Met1606 (located in the A2 domain of VWF). Exosites present in the spacer domain (3.), cysteine-rich domain (4.), and disintegrin-like domain (5.) regulate VWF substrate recognition. Binding of the disintegrin-like domain to VWF will also activate the otherwise latent metalloprotease domain, giving VWF access to the active-site cleft in ADAMTS13, which will eventually result in the proteolysis of VWF (6.).

immunodeficiency virus, pregnancy, cancer, drugs [ticlopidine and clopidogrel]).<sup>46–48</sup>

**Immune-Mediated TTP**

In iTTP, patients have developed an immune response against ADAMTS13.<sup>38</sup> However, why suddenly autoimmunity against ADAMTS13 appears and how ADAMTS13 deficiency is precisely induced are not well understood, but both genetic as well as environmental factors have been mentioned.<sup>49</sup> Human leukocyte antigen (HLA)-DRB1\*11 is a well-established predisposing factor for iTTP, besides female sex (2–3.5F/1M), obesity, and black ethnicity.<sup>1,7,21,50</sup> In Caucasians, the HLA-DRB1\*11 and HLA-DQB1\*03:01 alleles were found to be overrepresented among iTTP patients, whereas HLA-DRB1\*04 was protective.<sup>50–53</sup> A recent Italian study also suggested an association of the rs6903608 variant and HLA-DQB1\*05:03 with acquired TTP.<sup>54</sup> Interestingly, HLA-DRB1\*11 and HLA-DRB1\*03 positive donors could especially present the CUB2-derived peptides, FINVAPHAR and ASYLIRD, respectively, on dendritic cells or antigen-presenting cells (APC),<sup>55,56</sup> suggesting that carriers of those HLA genotypes could indeed be more prone to develop anti-ADAMTS13 autoantibodies and iTTP.

Molecular mimicry between a pathogen (*Influenza A*, *Helicobacter pylori*, *Brucella*, *Legionella*,...) and ADAMTS13

also been proposed as a possibility to evoke an immune response against ADAMTS13.<sup>57,58</sup>

**Anti-ADAMTS13 Autoantibodies**

To unravel the pathophysiology of iTTP, the anti-ADAMTS13 autoantibodies in iTTP patients have been a major source of investigation.<sup>59–64</sup> In plasma of iTTP patients, variable anti-ADAMTS13 autoantibody levels have been detected, indicating that even little amounts of autoantibodies can already induce ADAMTS13 deficiency.<sup>61</sup> Epitope mapping studies, using several ADAMTS13 fragments, revealed that the immune response in iTTP patients is polyclonal, since anti-ADAMTS13 autoantibodies have been found against all domains of ADAMTS13.<sup>59–66</sup> However, the spacer domain has been identified as an immunogenic region in ADAMTS13, since anti-spacer autoantibodies are present in the majority of the iTTP patients.<sup>59–65</sup> Anti-ADAMTS13 autoantibodies are classified into inhibitory (neutralizing ADAMTS13 proteolytic activity) and noninhibitory (binding to the protease and accelerating its clearance from plasma) autoantibodies.<sup>38,67–69</sup> Although inhibition was widely accepted as the major cause of ADAMTS13 deficiency, novel findings show that antigen depletion significantly contributes to ADAMTS13 deficiency.<sup>64</sup> Interestingly, also anti-ADAMTS13 autoantibodies which cause an open ADAMTS13 in iTTP patients have recently been identified.<sup>70,71</sup>

However, further research is needed to unravel the role of those “opening” antibodies in the pathophysiology of iTTP. Also the isotype class or subclasses of the anti-ADAMTS13 autoantibodies have been investigated, in which anti-ADAMTS13 autoantibodies are mainly of the immunoglobulin G (IgG) isotype (with IgG4 being the most common, followed by IgG1), but in 20% of the patients also IgAs and IgMs are found.<sup>65,68,72–75</sup> High IgA anti-ADAMTS13 antibody titers were associated with a worse prognosis as they were correlated with lower platelet counts, an increased severity, and mortality.<sup>72–74</sup>

Currently, also more than 90 monoclonal anti-ADAMTS13 autoantibodies isolated from iTTP patients have been cloned using phage display, Epstein–Barr virus immortalization, or B cell sorting followed by antibody cloning to further understand the immune response in iTTP patients.<sup>76–81</sup> Remarkably, almost all iTTP patients have VH1–69 encoded anti-ADAMTS13 autoantibodies and most of them target the spacer domain.<sup>57,76–80</sup> However, the role and function of anti-ADAMTS13 autoantibodies outside the spacer domain is largely unexplored.

### Immune Complexes

While almost 100% of the acute iTTP patients have free anti-ADAMTS13 autoantibodies, ADAMTS13-specific immune complexes were found in 30 to 97%.<sup>82–84</sup> Interestingly, also elevated C3a and C5a complement factors have been found during acute iTTP, which could either suggest complement activation through ADAMTS13 antigen–antibody immune complexes (classic pathway) or through the alternative pathway.<sup>58,82,85</sup> Remarkably, ADAMTS13-specific immune complexes were also found in 93% of the patients during remission.<sup>83</sup> It is thought that immune complexes are quickly cleared from circulation, but exceeding the clearance capacity could cause build-up of immune complexes and lead to a proinflammatory state.<sup>86</sup> Until now, it is not yet clear if those immune complexes and the complement system play a major role in the etiology of iTTP.

### Congenital TTP

cTTP is also rare and its prevalence uncertain (~0.5–2 cases per million people).<sup>87</sup> cTTP is linked to compound heterozygous or homozygous mutations spread over the complete *ADAMTS13* gene,<sup>15,22,45,87–92</sup> including approximately 200 different mutations in more than 150 patients.<sup>22,45,90,93–95</sup> In most cases, the mutations lead to secretion deficiencies, but they can also influence ADAMTS13 activity.<sup>41,93,96,97</sup> Additionally, also single nucleotide polymorphisms (SNPs) can influence ADAMTS13 secretion and activity and especially the interplay between different mutations and/or SNPs can affect ADAMTS13.<sup>41,93,96,98</sup> The first episode of cTTP typically occurs during the neonatal period or early childhood (before age 10),<sup>22,99</sup> but can also be evoked during pregnancy and is then often associated with a missense mutation in exon 24 (p.R1060W mutation),<sup>49,100,101</sup> The severity of the disease is also variable and probably related to specific *ADAMTS13* mutations and to additional genetic or environmental factors.<sup>22,45,87,90–92</sup>

## Diagnosis of TTP

### Clinical and Biological Suspicion of TTP

A severe thrombocytopenia (typically  $<30 \times 10^9/L$ ) and a microangiopathic hemolytic anemia (schistocytes on the blood smear) are the almost constant signs of TTP, often associated with corresponding symptoms (as skin or mucosal hemorrhage, weakness, dyspnea) and multivisceral ischemic symptoms targeting mainly the brain, mesenteric vessels, and the heart.<sup>6,102</sup> An elevated cardiac troponin-I (cTnI) level on admission is an independent predictor of death or refractoriness to treatment and represents a prognostic indicator in TTP patients.<sup>103,104</sup>

However, these clinical features are not specific for TTP, and distinguishing TTP from other disorders can be challenging. The main differential diagnosis of TTP are other TMA syndromes (as typical and atypical hemolytic uremic syndrome, associated to either Shigatoxin-producing *Escherichia coli* or abnormalities of the alternative complement pathway), TMA syndromes associated with pre-existing conditions (cancer, organ or hematopoietic stem cell transplantation, sepsis, pregnancy, ...), hematological cytopenia (Evans syndrome, isolated thrombocytopenia, or hemolytic anemia), ischemic manifestations linked to autoimmune disorders (lupus, immune thrombocytopenia, antiphospholipid syndrome...), or disseminated intravascular coagulation.<sup>102,105</sup> Moreover, older iTTP patients usually have several comorbidities and atypical neurological symptoms, leading to a delay in diagnosis.<sup>106,107</sup>

Two clinical scores (French score and PLASMIC score), derived from standard parameters at presentation, are reliable to identify patients with a severe ADAMTS13 deficiency who are most likely to benefit from therapeutic plasma exchange (TPE) in emergency before the results of ADAMTS13 activity become available.<sup>108,109</sup>

### ADAMTS13 Investigation

Since 1998, several in-house and commercial assays have been developed for ADAMTS13 investigations.

- *ADAMTS13* activity measurement is the first test to be performed in patients with a clinical suspicion of TMA. A severe ADAMTS13 deficiency (activity  $<10\%$ ) supports the diagnosis of TTP.<sup>37</sup> A normal or an only partial deficiency in ADAMTS13 activity differentiates other TMAs or conditions from TTP and reflects enzyme degradation, decreased synthesis, and secretion or catalytic inhibition of the protease. Assays are based on the degradation of a substrate (VWF full-length or synthetic peptide of VWF containing the cleavage site of ADAMTS13) by ADAMTS13 of the tested plasma sample, and the detection of VWF cleavage products by electrophoresis, platelet aggregation, fluorescence resonance energy transfer (FRET), or enzyme-linked immunosorbent assays (ELISAs).<sup>14,105,110–115</sup> In-house assays using full-length VWF and FRET-VWF73 are considered as reference methods for ADAMTS13 activity measurement, ideally calibrated against the new World Health Organization International Standard ADAMTS13 plasma ( $N = 50–150\%$ ).<sup>110–112,114,116</sup> However, these methods are

not fully automated and limited to expert laboratories, which can result in a time delay of 4 to 6 days before the ADAMTS13 activity results are available. For a few years, commercial FRET and chromogenic assays have been available,<sup>115,117-121</sup> and the first fully automated chemiluminescence immunoassay has recently been developed.<sup>121,122</sup> Additionally, also a semiquantitative ADAMTS13 activity assay has recently been released.<sup>123</sup>

- *The identification of anti-ADAMTS13 autoantibodies* is the second step of investigation to document the mechanism of ADAMTS13 deficiency.<sup>38,39</sup> Inhibitory ADAMTS13 autoantibodies are screened in vitro using mixing assays of heat-inactivated plasma samples at several dilutions, and Bethesda assays.<sup>124</sup> Anti-ADAMTS13 IgGs are also titrated in vitro using different ELISA methods (in-house or commercial assays, performed in a specialized laboratory), using different coated ADAMTS13 antigens and methods of detection.<sup>38,39,72,125</sup>
- *ADAMTS13 antigen level* is less important in the diagnosis of TTP, but it has recently been linked to the outcome of iTTP patients and therefore could be useful to measure.<sup>126</sup> In cTTP, measuring ADAMTS13 antigen levels is used for the documentation of ADAMTS13 deficiency and is dependent on the type of mutation. Different monoclonal and polyclonal antibodies are available to quantify antigen levels using in-house or commercial ELISAs.<sup>69,70</sup>
- *Sequencing ADAMTS13 gene* (NM\_139025.4) aims at documenting sequence variations to confirm cTTP, in selected patients.

### Treatment of Thrombotic Thrombocytopenic Purpura

TTP remains a life-threatening disease with a mortality rate of 10 to 20% and a relapsing tendency in spite of appropriate therapeutic management in patients usually admitted at the intensive care unit (ICU).<sup>6,10,127</sup> The recent advances in the treatment of TTP have identified ADAMTS13, anti-ADAMTS13 autoantibodies, and VWF as the three therapeutic targets.<sup>128-132</sup>

### Plasma Therapy

In 1991, Rock et al empirically used intensive TPE improving the management of acute TTP episodes.<sup>133</sup> Today, TPE is the established front-line treatment of the acute phase and should be started as soon as the diagnosis of TTP is established or strongly suspected until complete remission (platelet count above  $150 \times 10^9/L$  for 2 consecutive days, together with normal or normalizing lactate dehydrogenase and clinical recovery).<sup>7,8,133</sup> In iTTP, daily TPE is more efficacious than plasma infusion, supplying ADAMTS13 and also removing circulating anti-ADAMTS13 autoantibodies and UL-VWF. Twice-daily TPE could be considered in refractoriness (absence of response to treatment) or exacerbation of TTP (early recurrence of iTTP within 30 days of treatment response).<sup>134,135</sup> However, immunomodulating agents are needed to definitely suppress the autoimmune component of iTTP. In cTTP, plasma infusions are usually sufficient to supply ADAMTS13 during the acute phase and prophylactic plasma infusions every 2 to 4 weeks are appropriate for patients with a chronic disease.<sup>22,87,90</sup>

### Corticosteroids

Corticosteroids are usually associated to TPE as immunosuppressant drugs, given the autoimmune nature of iTTP. Corticosteroids may also reduce the number of TPE and improve the tolerance of plasma therapy, despite the paucity of high-quality evidence-based studies.<sup>136-138</sup>

### Rituximab

Rituximab, a humanized anti-CD20 monoclonal antibody, is used in association with TPE, to induce a depletion of peripheral B cells and to block the production of anti-ADAMTS13 autoantibodies, with a 10 to 14-day delay to be efficient in TTP.<sup>139-145</sup> Rituximab was first introduced periodically in iTTP patients with a suboptimal response to the standard treatment (four infusions of rituximab  $375 \text{ mg/m}^2$ , once or twice weekly). Recently, frontline therapy with rituximab reported shorter hospitalization and time to treatment response, fewer relapses, but no benefit on early deaths in iTTP patients.<sup>143,145,146</sup> Preemptive rituximab is still considered in iTTP patients with a persistent or recurrent ADAMTS13 severe deficiency to prevent relapse. Although the preemptive effect of rituximab reduces over time, the use of rituximab is associated with a lower mortality during follow-up.<sup>142,146-154</sup> Recent studies also investigated whether the doses of rituximab can be reduced, since multiple infusions of rituximab may expose patients to infection or other long-term complications although treatment is generally well tolerated.<sup>155,156</sup>

### Other Immunomodulatory Drugs

Vincristine, cyclosporine A, cyclophosphamide, or mycophenolate mofetil are used as a third-line option, in patients with a refractory iTTP to stop the production of anti-ADAMTS13 autoantibodies.<sup>129,157-160</sup> Bortezomib, a proteasome inhibitor inducing depletion of residual autoreactive and pathogenic B-cells and plasma cells, has recently been reported in a few cases to induce remission in refractory iTTP.<sup>161,162</sup> Splenectomy is considered and has been shown to be successful in more severe patients with a relapsing or refractory autoimmune TTP who do not respond to other therapies.<sup>163-165</sup>

### Caplacizumab

Caplacizumab (ALX-0081) is a nanobody derived from single-chain antibodies naturally occurring in *Camelidae*. Caplacizumab targets the A1 domain of VWF and blocks the interaction of VWF multimers with platelets. The potential of caplacizumab became evident in a safety and efficacy study using a baboon model for TTP, in which a fast platelet recovery without a severe bleeding risk was observed.<sup>166,167</sup> Multicenter randomized placebo-controlled clinical trials TITAN (phase II) and HERCULES (phase III) have shown that caplacizumab administration after each TPE and for 30 days thereafter (or treatment extension for up to 4 weeks more, in cases of persistent ADAMTS13 deficiency) may shorten the hospitalization stay, the number of TPE sessions, and the number of days until treatment response, thereby improving preservation of organ integrity and survival in iTTP patients. Caplacizumab presents a good safety profile and minor bleedings, considered manageable, are reported.<sup>168,169</sup> Caplacizumab, recently approved

in both Europe and United States, provides a therapeutic bridge to effective immunosuppression in iTTP therapeutic management.<sup>168–174</sup>

### rADAMTS13

In cTTP, recombinant ADAMTS13 (rADAMTS13; SHP655, BAX930) was evaluated in a phase I study and considered safe and well tolerated, with a half-life of 53 hours,<sup>24,175,176</sup> similar to that reported for endogenous ADAMTS13 in plasma. After rADAMTS13 infusion, platelet counts increased and UL-VWF decreased in cTTP patients. Currently, a phase III randomized controlled trial is ongoing in cTTP (<https://clinicaltrials.gov/ct2/show/NCT03393975>). Since administration of rADAMTS13 in iTTP patient plasma and in a rat model for iTTP could neutralize circulating anti-ADAMTS13 autoantibodies thereby restoring ADAMTS13 activity,<sup>175,177,178</sup> rADAMTS13 will also be evaluated in a phase II randomized controlled study in iTTP, by saturating anti-ADAMTS13 antibodies and cleaving UL-VWF (<https://clinicaltrials.gov/ct2/show/NCT03922308>).

### N-Acetylcysteine

N-Acetylcysteine (NAC), a Food and Drug Administration–approved mucolytic agent, inhibits platelet adherence to endothelial cell-anchored soluble UL-VWF by reducing their size. NAC also reduces intramolecular VWF–disulfide bonds as in its A1 domain, which contains the binding site of VWF for platelet glycoprotein Ib $\alpha$  (GPIb $\alpha$ ).<sup>179–183</sup> Although prophylactic administration of NAC was able to prevent severe TTP signs in mice, NAC treatment was not able to resolve severe TTP signs in mice or baboons.<sup>183</sup> Currently, the use of very high doses of NAC as an adjunct therapy of TTP is investigated in a phase I trial to assess its potential efficacy in TTP (<https://clinicaltrials.gov/ct2/show/NCT01808521>).

## Long-Term Outcomes and Follow-up

### Relapses

iTTP patients relapse when they are experiencing a severe decrease in ADAMTS13 activity by the persistence or the recurrence of anti-ADAMTS13 autoantibodies more than 30 days after stopping TPE.<sup>72</sup> Clinical relapses are associated to a risk of death, TPE complications, or ICU hospitalization,<sup>127</sup> and their prevention is a concern. Especially patients with a persistently low ADAMTS13 activity should avoid external triggers (infections, pregnancy, surgery) to avoid evoking an acute iTTP bout. In iTTP, the use of rituximab at the acute phase decreased the relapse rate up to 1 year, but this effect diminishes over time.<sup>151,153</sup> Considering a persistent severe ADAMTS13 deficiency as a risk factor for relapse, preemptive rituximab should be considered to restore ADAMTS13 activity together with peripheral B cell depletion.<sup>126,148,150</sup>

In cTTP, some patients are dependent on regular prophylactic plasma infusions every 2 or 3 weeks. Prophylactic plasma therapy is also required during pregnancy to avoid a TTP episode.<sup>22,87</sup>

### Long-Term Follow-up

In iTTP, the occurrence of biological or clinical autoimmunity and late relapses are reported many years following the recovery.<sup>2,7,8,184</sup> Long-term follow-up of iTTP patients is based on standard biology, ADAMTS13 activity monitoring, and medical consultations to detect emerging autoimmune diseases (systemic lupus erythematosus, Gougerot–Sjögren syndrome, connective tissue diseases), to evaluate cognitive disturbances, arterial hypertension, and major depression.<sup>5,8</sup>

In cTTP, long-term follow-up is important and outcomes are poorly reported (chronic kidney disease, stroke, cardiac ischemia, death).<sup>22,45,87,90</sup>

## Open Questions

### Unmet Needs, Gaps of Knowledge, Areas of Debate, and Controversies

#### Why Do Some Patients Not Relapse Despite a Persistent Severely Deficient ADAMTS13 Activity in Remission?

In clinical remission, a persistent severe ADAMTS13 deficiency predicts a risk of relapse.<sup>150,185</sup> However, a severe ADAMTS13 deficiency is not sufficient to induce a TTP episode and additional triggers are required and need to be identified. Some conditions increasing plasma VWF levels such as inflammation, sepsis, or pregnancy are known to potentially act as precipitating factors of acute TTP episodes. Recently, a reduced DNase activity in TTP patient plasma was shown to result in the persistence of neutrophil extracellular traps (NETs) and could serve as the second hit needed to evoke an acute TTP bout, since human neutrophil peptides are also able to inhibit ADAMTS13 activity in vitro.<sup>186–188</sup>

#### What Is the Mechanism Leading to the Loss and Re-establishment of Self-Tolerance of the Immune System toward ADAMTS13?

Genetic (gender, ethnicity, HLA haplotype) and/or environmental (infection, stress, surgery, obesity) factors have been mentioned to contribute to the onset of the disease.

Autoreactivity can appear when low-affinity autoreactive CD4+ T cells escape the negative selection in the thymus and recognize an antigen presented on an APC through an HLA molecule.<sup>58</sup> Genetic factors such as HLA-DRB1\*11 in Caucasians<sup>51,75,189</sup> are predisposing factors for iTTP. Interestingly, especially HLA-DRB1\*11 and HLA-DRB1\*03 positive donors could present CUB2-derived peptides (FINVAPHAR and ASYLIRD) on dendritic cells or APC,<sup>55</sup> while reactive CD4+ T cells against those peptides have been identified in iTTP patients,<sup>56,189</sup> suggesting that stimulation of low-affinity self-reactive CD4+ T cells might play a role in the development of anti-ADAMTS13 autoantibodies. Other factors including hormones, cytokines, and other mediators may play a role in the loss of self-tolerance and the initiation of the immune response toward ADAMTS13.

Infections have been found to be an underlying cause for triggering an acute iTTP episode. Therefore, it has been

suggested that molecular mimicry between the pathogen and ADAMTS13 could be responsible for the development of reactive anti-ADAMTS13 autoantibodies causing iTTP.<sup>58</sup>

Further studies are required to elucidate these mechanisms leading to a loss of self-tolerance.

#### What Is the Role of the Open ADAMTS13 Conformation in the Loss of Self-Tolerance and Development of Anti-ADAMTS13 Autoantibodies?

Exposure of cryptic epitopes can lead to the development of autoantibodies. Recently it has been shown that iTTP patients have an open ADAMTS13 conformation and anti-ADAMTS13 autoantibodies against cryptic epitopes in ADAMTS13 have been identified.<sup>70,81,190</sup> It has been suggested that the development of anti-ADAMTS13 autoantibodies could be caused by exposure of cryptic epitopes due to interaction of ADAMTS13 with certain drugs, since several drugs (ticlopidine, clopidogrel) have been associated with the onset of iTTP.<sup>46–48</sup> However, also other mechanisms like posttranslational modifications (glycosylation, oxidation, citrullination...) could lead to the exposure of cryptic epitopes.<sup>191</sup> Indeed, changes in N-linked glycans in the CUB domains resulted in the exposure of a cryptic epitope in the metalloprotease domain in vitro.<sup>191</sup> Interestingly, also anti-ADAMTS13 autoantibodies purified from iTTP patients are able to change the ADAMTS13 conformation.<sup>71</sup> However, whether this open ADAMTS13 is the initial step in the loss of self-tolerance and the development of anti-ADAMTS13 autoantibodies or whether it is a consequence of the immune response needs further investigation.

#### Why Do Some Patients Frequently Relapse despite an Appropriate Immunosuppressive Therapy or Why Are Some Patients Unresponsive to Rituximab?

Despite the same therapeutic management, some patients have a refractory iTTP to standard treatment, or relapse after reaching treatment response. Some patients are also unresponsive to rituximab and the mechanisms by which B cells resist to rituximab remain unclear. The hypothesis is based on resistance to complement-dependent cytotoxicity, to antibody-dependent cellular toxicity, to apoptosis and downregulation, or modulation or loss of CD20 on B cells by transformation into plasma cells.<sup>192</sup>

#### How to Manage cTTP Patients with the p.R1060W Mutation in a Non-obstetrical Context?

The mutation p.R1060W (c.3178C > T) in the TSP1–7 domain of ADAMTS13 is reported with a high prevalence in adult-onset cTTP, mostly in women during their first pregnancy,<sup>22,87,101,193</sup> and some cases have been reported in men.<sup>100,194</sup> The p.R1060W mutation is associated with a residual ADAMTS13 activity, supporting the absence of a TTP episode during childhood for most patients. However, some conditions are precipitating factors of acute TTP by increasing plasma VWF levels, as an inflammatory context or surgery for example, and there are no guidelines on how to manage these conditions.

#### Are the Diagnosis Algorithms Sufficiently Reliable to Initiate a Frontline Treatment with TPE, Rituximab, and Caplacizumab? How to Improve a Rapid Diagnosis of TTP?

TTP is still underdiagnosed and challenging by its rarity and non-specificity of clinical features at presentation. In the absence of rapid turnaround assays for ADAMTS13 activity measurement, clinical scores are calculated, but are they reliable enough to initiate a treatment with TPE, rituximab, and caplacizumab?<sup>108,109</sup> The prompt availability of ADAMTS13 activity measurement could be helpful to adjust the front-line treatment to the severity of the disease. Recently the first fully automated chemiluminescence immunoassay for the measurement of ADAMTS13 activity in citrated human plasma has been developed and evaluated by the Italian group with promising results.<sup>121,122</sup> Additionally, a semiquantitative ADAMTS13 activity assay has recently been released to make ADAMTS13 activity measurements directly available, and is under evaluation in several countries.<sup>123</sup>

#### What Is the Place of Emerging Treatment Strategies in the Management of iTTP?

From the 1990s, the standard treatment of TTP patients involved TPE and additional immunosuppression. In the 2000s, rituximab has been used as an additional treatment to TPE, which reduces the number of TPE procedures and additionally increases relapse-free survival.<sup>143</sup> Recently, the use of rituximab as a frontline therapy, together with TPE and steroids, was reported by the French, UK and U.S. groups.<sup>143,144,195</sup> However, the minimal effective dose of rituximab should be defined in a clinical trial (<https://clinicaltrials.gov/ct2/show/NCT01554514>).<sup>130,131,196</sup> Preemptive rituximab could also be used in remission when ADAMTS13 activity drops below 10%, to avoid iTTP relapses. However, preemptive rituximab is debatable among physicians and there is no international consensus.

Caplacizumab is a new target therapy, used at the acute phase of iTTP and for 30 consecutive days after the cessation of TPE. After this, the use of caplacizumab can be extended in patients with a persistent severely deficient ADAMTS13 activity. The management of caplacizumab therapy with the monitoring of ADAMTS13 activity and the possibility of home therapy are attractive for the management of TTP patients. Further studies are required to determine the optimal time for stopping caplacizumab when ADAMTS13 is no longer severely deficient (activity >20%) and its use to prevent TTP relapses. Whether a combined therapy of rituximab and caplacizumab without the need of TPE will be sufficient to treat TTP patients also needs further investigation. The high cost of this treatment is a concern.

#### Ongoing Research

##### Therapeutic Management of iTTP

After the introduction of TPE 30 years ago, the management and survival of acute TTP patients improved significantly. However, in the next few years, novel targeted therapies, such as low-dose rituximab, caplacizumab, rADAMTS13, and NAC, may change the empiricism of TPE, thanks to their evaluation by ongoing clinical trials and pilot studies.

Bortezomib and rituximab could be synergistic in refractory iTTP. In iTTP, rADAMTS13 could be tested together with novel approaches using genetically engineered ADAMTS13 variants to escape the inhibition by anti-ADAMTS13 autoantibodies,<sup>197</sup> or plasmin to bypass ADAMTS13.<sup>198,199</sup> Also other approaches like the use of high doses of magnesium sulfate (<https://clinicaltrials.gov/ct2/show/NCT03237819>), Microlyse (fusion protein between a nanobody and an enzyme that breaks down microthrombi), efgartigimod and rozanolixizumab (antibodies that block FcRn–IgG interaction inhibiting IgG recycling and inducing IgG clearance), or Ides<sup>200</sup> are currently under investigation to further improve the management of TTP patients.

*Adamts13*<sup>-/-</sup> mice models have been used to test different gene therapy strategies to cure cTTP, in which *ADAMTS13*-encoding lentiviral vectors, adenoviral vectors, or a non-viral sleeping beauty transposon system were used for the expression of ADAMTS13 to cure cTTP.<sup>201–204</sup> Therefore, gene therapy may have a role in the future management of cTTP.<sup>203,205</sup>

### TTP Pathophysiology

The fibrinolytic enzyme plasmin cleaves ADAMTS13, reduces its activity in vitro, and is also able to cleave VWF. In iTTP patients, endogenous plasmin levels are increased during the acute phase and potentially involved during a TTP episode. In mice, administration of plasmin resolves TTP features, increasing the proteolysis of VWF.<sup>198,199</sup>

Endothelial injury, acute inflammation, or infection may trigger the formation of microvascular thrombosis in patients with iTTP. Human neutrophil peptides 1–3 (HNPs1–3) released from activated and degranulated neutrophils inhibit proteolytic cleavage of VWF. HNPs1–3 may inhibit ADAMTS13 activity, suggesting a link between inflammation, infection, and formation of microvascular thrombosis in iTTP.<sup>186,188</sup> On top of that, NETs have been found in TTP patient plasma.<sup>186</sup> The activation of the complement alternative pathway was reported in iTTP patients and further studies have to be performed to understand the implication of the complement pathway and potential therapeutic issues.<sup>206–208</sup> However, an increased complement activation was observed in patients who died during the acute episode compared with those who achieved remission.<sup>209</sup>

Other still unknown epigenetic changes in gene expression, posttranslational modifications related to environmental influences, should also be further explored.

### Open ADAMTS13 Conformation as a Biomarker for iTTP

It has been shown that an open ADAMTS13 conformation is a unique hallmark of iTTP patients.<sup>70</sup> Recently it has been shown that this unique hallmark is not only present during the acute phase, but also during remission even before a severe drop in ADAMTS13 activity (<10%).<sup>71</sup> This shows that patients should be closely monitored. However, whether the open ADAMTS13 conformation has a predictive value and whether patients in remission with an open conformation before a severe drop in ADAMTS13 activity (<10%), indicating presence of anti-ADAMTS13 autoantibodies, should be treated preemptively should be further investigated.

### Use of ADAMTS13 Variants as Therapy

The immune response in iTTP patients is polyclonal, since anti-ADAMTS13 autoantibodies against all domains in ADAMTS13 have been identified.<sup>59–65</sup> However, the majority of iTTP patients contain anti-ADAMTS13 autoantibodies which target the spacer domain.<sup>59–65</sup> The spacer domain plays an important role in both containing the closed conformation and the interaction with VWF. Therefore, it is under investigation whether circumventing anti-ADAMTS13 autoantibody binding to the spacer domain, by making an ADAMTS13 spacer variant, could be a novel therapeutic strategy.<sup>65,197,210,211</sup> Therefore, also large epitope mapping studies and further characterization of the anti-ADAMTS13 autoantibodies are needed, since the functional significance of autoantibodies targeting domains outside the spacer domain has been largely unexplored. The potential of this strategy will depend on the diversity of (harmful) anti-ADAMTS13 autoantibodies outside the spacer domain in the iTTP patients.

### Time Capsule

#### Rapid Turnaround Assay for the Diagnosis of TTP

Implementation of simple assays for ADAMTS13 activity, antigen, autoantibodies, and conformation measurement will be helpful to diagnose TTP in emergency and to adjust the frontline treatment to the severity of iTTP.

#### Frontline Treatment of iTTP

Frontline treatment of iTTP might change in the next few years, and will be defined by the triplet combination of rADAMTS13, rituximab, and caplacizumab, supplemented with corticosteroids.

#### ADAMTS13 Conformation

Besides ADAMTS13 activity, anti-ADAMTS13 autoantibodies, also ADAMTS13 conformation might become an important parameter to correctly diagnose iTTP and could be implemented in the follow-up of iTTP patients.

#### Loss of Self-Tolerance and Immune Response in iTTP

Looking at the advances that have been made in the understanding of the pathophysiology of iTTP the past few years, future research will be able to unravel the mechanism of the loss of self-tolerance and the development of an immune response against ADAMTS13 in iTTP patients.

### Conclusion

The fundamental understanding, diagnosis, and treatment of TTP have been majorly advanced during the last decades. Currently, the initial diagnosis of TTP is based on the use of clinical scores, but ADAMTS13 activity <10% is the unique marker which will identify TTP. Several targeted therapies (rituximab, caplacizumab, rADAMTS13) are under investigation and have improved the management of TTP patients and their quality of life. However, still a lot of questions remain unanswered to completely understand this rare and

devastating disease. Therefore, future research is still needed to further increase our knowledge on TTP.

#### Conflict of Interests

The authors declare that they have no conflict of interest.

## Authors



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Dr. Elien Roose graduated as Master of Science in Biochemistry and Biotechnology at the KU Leuven (Leuven, Belgium) and obtained her PhD in Biochemistry in 2018 in the Laboratory for Thrombosis Research at the KU Leuven

Campus Kulak Kortrijk, Belgium, under the supervision of Prof. Karen Vanhoorelbeke, where she is now continuing her research as a postdoc. Her research is focused on investigating the folding of ADAMTS13 and the role of anti-ADAMTS13 autoantibodies to create novel insights in the pathophysiology of the rare and life-threatening autoimmune disorder immune-mediated thrombotic thrombocytopenic purpura (iTTP). She had the opportunity to meet many TTP experts from Europe and has a close ongoing collaboration with Prof. Agnès Veyradier, Prof. Paul Coppo, and Dr. Bérange S. Joly, who run the French Reference Center for TMA.



### Bérange S. Joly

Dr. Bérange S. Joly is a PharmD, PhD, and medical biologist working at the Lariboisière hospital, teaching and doing research in hemostasis and thrombotic microangiopathic (TMA) syndromes. Her expertise lies in hemostasis, in

the diagnosis of TMA syndromes, and in understanding thrombotic thrombocytopenic purpura (TTP) pathophysiology. She obtained her PhD in hematology in 2018 in the EA3518, *Institut de Recherche Saint-Louis*, Saint-Louis hospital, at Paris Diderot University, supervised by Prof. Agnès Veyradier. She had the opportunity to work on ADAMTS13 conformation in collaboration with Dr. Elien Roose and Prof. Karen Vanhoorelbeke in the Laboratory for Thrombosis Research in Kortrijk, in Belgium. Now she is a postdoc working in the field of immune-mediated TTP pathophysiology and autoimmune diseases. She also wants to contribute to improve the quality of life of TTP patients, TTP diagnosis, and management on behalf of the French Reference Center for TMA.

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